Covaris truCOVER® Library Prep: A Streamlined WGS Workflow for Enhanced NGS Library Performance and Data Quality

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Introduction

NGS Library Prep: Challenges & Solution

- As WGS gets more widely adopted, optimizing workflows to address fragmentation inconsistencies, poor library conversion, input variability, and sequencing bias is critical for accurate and scalable variant reporting. [1]
- For low input material, conventional amplification techniques often introduce biases, leading to non-uniform coverage, preferential amplification of regions with extreme GC content, and a subsequent compromise in data quality and variant calling precision. [2]
- Covaris truCOVER Library Prep offers precise, mechanical DNA fragmentation along with optimized reagents and workflow to address all above challenges.

truCOVER Benefits

Key Benefits

- **Operational Efficiency:** Streamlined workflow with integrated PCR-free and amplification options saves time and labor.
- **Broad Compatibility:** Works with diverse sample types (blood, saliva, FFPE, cell cultures, microbes) and input ranges (0.1–500 ng).
- **Reliable Performance:** Consistent DNA shearing across samples; minimal bias even at low input (down to 100 pg).
- **Fast & Scalable:** Prep completed in <3 hours with only 25 minutes hands-on; supports up to 96 samples per kit.
- **High-Quality Output:** Delivers uniform, reproducible libraries—even from degraded DNA—with low duplication and no GC bias.
- **Cost-Effective:** Fewer steps and consistent results reduce reagent use, repeat runs, and sequencing waste.

truCOVER: Workflow

- Detailed protocols, including Covaris AFA® settings, library quantification procedures, and workflow specifics are described in the truCOVER Library Prep Kit product manual [3] and in the associated Application Note [1].

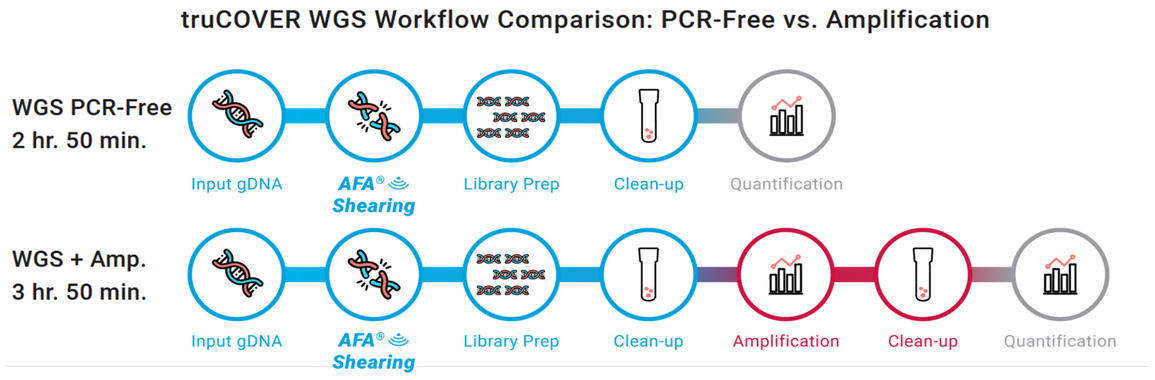

Figure 1. Workflow for truCOVER PCR-free vs Amplification modules.

Methods and Results

Library Prep

- **Library Prep & Amplification:** WGS libraries prepared using truCOVER Library Prep (PN 520361) and Amplification Kit (PN 520366).
- **DNA Sources:** Coriell NA12878 human reference DNA, Pseudomonas aeruginosa (ATCC PN27853, GC 66.1%), Staphylococcus aureus (ATCC PN25923, GC 32.3%).
- **FFPE Sample Processing:** Coriell FF12878 FFPE Scroll; gDNA extracted using truXTRAC FFPE Total NA Auto 96 Kit (Covaris, Cat. No. 520318). 200 bp was used during fragmentation.

Sequencing

- Coriell libraries were pooled equimolar based on mass (Qubit) and sequenced on a NovaSeq X Plus instrument to ~30x coverage. Bacteria libraries were sequenced to >200x coverage on a NextSeq 2000.
- Sequencing data was analyzed using the CLC Genomics Workbench (Qiagen).

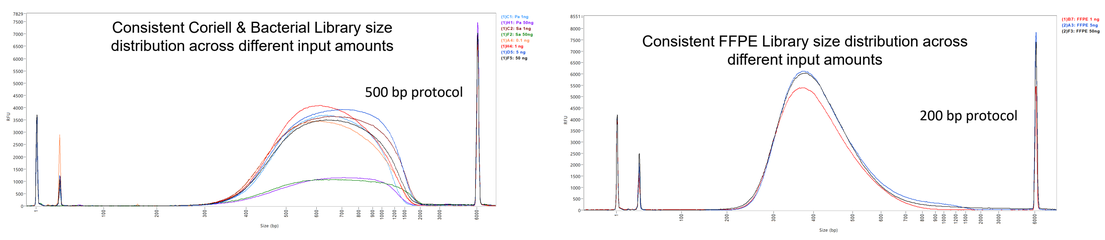

Figure 2. Fragment Analyzer traces for Coriell NA12878, P. aeruginosa, S. aureus (top), and Coriell FFPE (bottom) after library preparation with different input amounts.

Result Highlights

- Uniform fragment size across sample types and input levels. (**Figure 2**)
- GC-independent shearing enables consistent, linear amplification across diverse DNA sources regardless of GC content. (**Figure 3**)
- Compatible with Illumina platforms; libraries exceed 2 nM input threshold. # PCR cycles optimized for different input amounts: 0.1ng – 12 cycles, 1ng – 10cycles, 5ng – 8 cycles, 50ng – 4 cycles to ensure minimal amplification bias and duplication rates. (**Figure 4**)
- Consistent median insert size confirms reproducible performance. (**Figure 4**)
- Superior F1 scores for both INDEL and SNP detection, even with DNA inputs as low as 0.1 ng. (**Figure 5**)

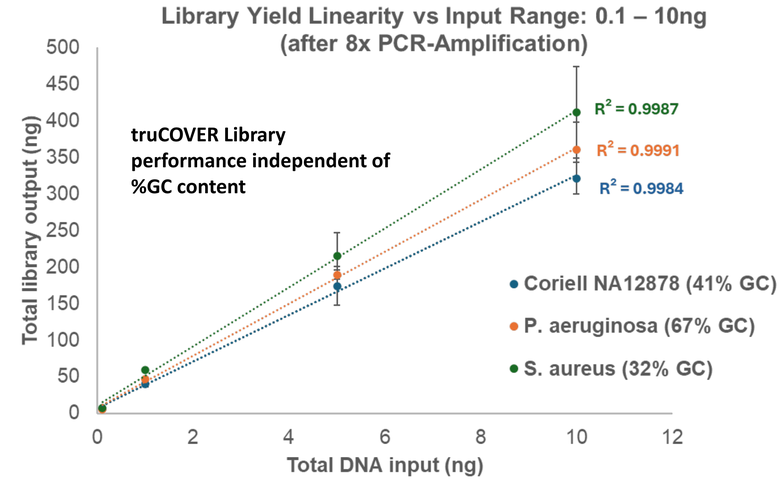

Figure 3. Libraries starting with 0, 1, 1, 5, and 10 ng of each of genomic DNA from Coriell NA12878 (41% GC), Pseudomonas aeruginosa (67% GC) and Staphylococcus aureus (32% GC), were generated using the truCOVER WGS PCR-free library kit workflow. The resulting size-selected library-converted DNA was directly amplified for 8 PCR cycles using the truCOVER Library Amplification Kit. Library yields were determined by Qubit. All libraries were done in triplicate.

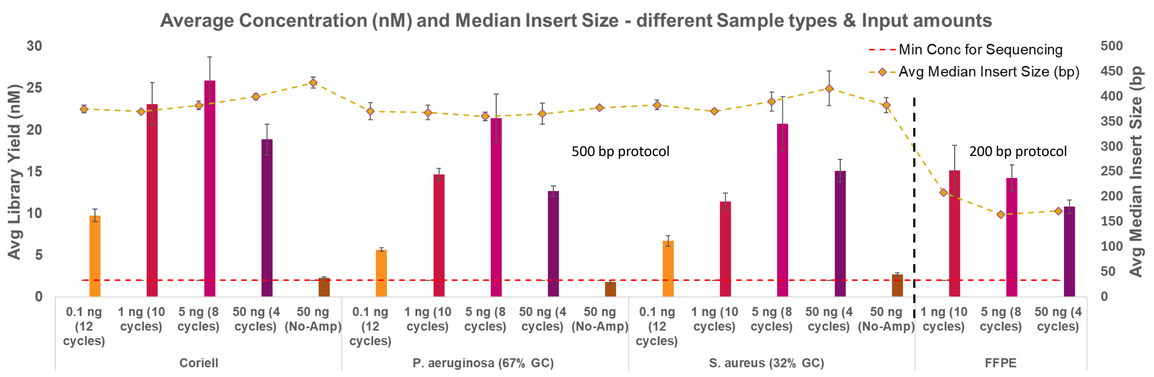

Figure 4. DNA concentration and Median Library Insert Size using the truCOVER Library Amplification workflow. Data includes sample types like Coriell NA12878, P. aeruginosa, S. aureus, and FFPE samples with different DNA input amounts ranging from 0.1ng, 1ng, 5ng, and 50ng. Data also includes no amplification controls with 50ng DNA input. Coriell NA12878, P. aeruginosa, S. aureus were sheared with 500bp protocol, while FFPE samples were sheared with 200 bp protocol.,

Sequencing Data

Coverage and Variant Calling Accuracy

- The truCOVER Amplification Kit demonstrably improves the quality of sequencing libraries.
- It demonstrates high coverage across a broad spectrum of input DNA concentrations, which directly correlates with more reliable and accurate variant calls in WGS and WES studies.

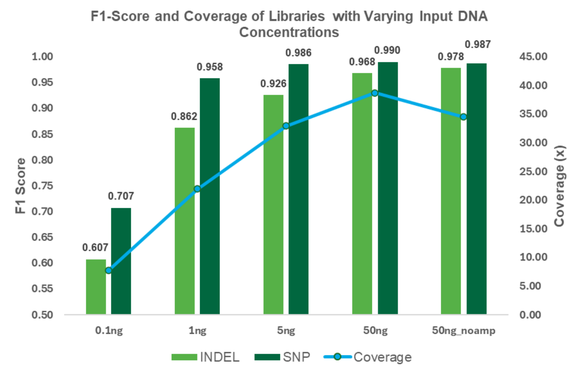

Figure 5. F1 score for INDEL and SNP detection, alongside the coverage (x-fold), of equimolarly pooled libraries of Coriell DNA (NA12878).

Conclusions

- The truCOVER workflow offers a simplified, modular approach with minimal manual intervention and faster turnaround time, boosting lab efficiency and scalability.
- The DNA amplification module delivers linear performance across varied GC content and sample types, ensuring reliable fragment sizing and reproducible insert lengths.

References

1. Ambavaram M, et al. Covaris truCOVER Library Prep Kit: A streamlined, PCR-free WGS workflow for enhanced NGS library performance and data quality. Covaris Application Note. 2025. https://www.covaris.com/wp/wp-content/uploads/resources_pdf/M020185.pdf

2. Quail, M., et al. (2024). Identifying the best PCR enzyme for library amplification in NGS. PMC - PubMed Central. https://pmc.ncbi.nlm.nih.gov/articles/PMC11092157/

3. Covaris. truCOVER DNA Library Prep Kits User Manual. 2025. Available at: https://www.covaris.com/wp/wp-content/uploads/resources_pdf/010673_User_Instructions_truCOVER_DNA.pdf

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